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Chondroprotective effects of high-molecular-weight cross-linked hyaluronic acid in a rabbit knee osteoarthritis model

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SUMMARY

Objectives: We hypothesized that high-molecular-weight (MW) cross-linked (CL) hyaluronic acid (HA) improves joint lubrication and has an enhanced chondroprotective effect. We examined the histopathological changes and friction coefficients in osteoarthritic knee joints after injecting high-MW CL HA.
Design: A bilateral anterior cruciate ligament transection (ACLT) model in 20 Japanese white rabbits was used. From week 5 after transection, low-MW HA (0.8×10^6 Da; HA80) or high-MW CL HA (6×10^6 Da; HA600) was injected weekly into 10 right knee for 3 weeks; normal saline (NS) was injected into the 10 left knee. A sham operation was undertaken to exclude spontaneous osteoarthritis (OA) in five knees. Results were evaluated with macroscopy, histopathology (Kikuchi's score), biomechanical testing, and rheological assessment of the joint fluid viscoelasticity. Statistical analysis was performed using one-way analysis of variance with a 95% confidence interval (CI) ($P < 0.05$).
Results: The macroscopic findings showed severely damaged cartilage in 30% of the NS group and 20% of the HA80 and HA600 groups and intact cartilage in 100% of the sham group. The histological scores and friction coefficients of the HA600 group were significantly lower than those of the NS group ($P = 0.007$ and $P = 0.002$, respectively). Viscoelasticity measurements of the joint fluid showed no significant differences between the three treatment groups.
Conclusion: High-MW CL HA exerts potential chondroprotective effects and produces superior friction coefficients. Our results suggest that HA600 delays the progression of OA effectively and improves joint lubrication significantly.

Introduction

Current treatments for osteoarthritis (OA) range from minimal nonsurgical measures to invasive and surgical modalities. Nonsurgical nonpharmacological measures include exercise, weight loss, and bracing; surgical measures vary from arthroscopic debridement and osteotomy to arthroplasty. Pharmacological therapies, such as analgesics, intra-articular injections, and topical treatments, mainly target the palliation of pain[1,2]. Chondroprotective agents are defined as compounds that (1) stimulate the synthesis of collagen and proteoglycans by chondrocytes and production of hyaluronic acid (HA) by synoviocytes; (2) inhibit cartilage degradation; and (3) prevent fibrin formation in the subchondral and synovial vasculature[3]. Compounds that show some of these characteristics are endogenous molecules of the articular cartilage, including HA, glucosamine, and chondroitin sulfate.

Viscosupplementation is an intra-articular therapeutic modality based on the physiological importance of HA in the synovial joints[4,5]. HA is a heteropolysaccharide formed by a variable number of repeating units of D-glucuronic acid and N-acetylglucosamine. It is formed by synoviocytes, fibroblasts, and chondrocytes inside joints and is present in the synovial fluid and the extracellular matrix of the cartilage. HA is crucial to the viscoelastic properties that allow the efficient movement of articular joints[6–9].

Several HA preparations are being marketed with varying molecular weights (MWs), ranging from 0.5×10^6 Da to 6×10^6 Da. Clinical trials have not indicated a clear advantage of one product over another[10]. One of the goals of HA therapy is to restore the viscoelasticity of the synovial fluid and the natural

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protective functions of HA in the joint. In humans, the MW of HA in a healthy knee joint is 6×10^6 Da. It has been reported that the MW of HA in a human arthritic knee joint decreases to $0.5–3 \times 10^6$ Da[11]. HA can potentially improve viscoelasticity, and a higher MW of HA is thought to improve the viscoelastic properties of HA preparations. However, the effects of high-MW cross-linked (CL) HA on joint viscoelasticity and joint lubrication have not yet been reported.

We hypothesized that high-MW CL HA improves joint lubrication and has an enhanced chondroprotective effect compared with lower-MW HA. We examined the histopathological changes in osteoarthritic knee joints after HA injection and investigated the relationship between the friction coefficient and the MW of HA.

Materials and methods

OA model

Twenty-three female Japanese white rabbits aged 12 weeks and weighing 2.6–2.9 kg were purchased from a professional breeding company (Japan SLC Inc., Hamamatsu, Japan) and used for this experiment. Our institutional Animal Care Committee approved the study, which was conducted according to its ethical guidelines and regulations. All animals were anesthetized with an intravenous injection of 0.05 mg/kg phenobarbital sodium, followed by gas anesthesia with isoflurane. Both knees of each rabbit were shaved, prepared, and draped in a sterile environment. To generate osteoarthritic joints, anterior cruciate ligament transection (ACLT) was performed in both knees using a medial parapatellar approach. After the patella was dislocated laterally, the knee was flexed maximally so that the anterior cruciate ligament could be readily visualized and identified. It was then transected with a #11 blade. The knee joints were inspected for bleeding and washed thoroughly with sterile normal saline (NS). The joint capsule was closed with a running suture of 4-0 nylon, and the skin incision was closed with running mattress sutures of 3-0 nylon[12]. Instability was confirmed in all knees with positive anterior drawer and Lachman tests. After surgery, the animals were allowed unrestrained cage activities while they were monitored for infections and other complications. To rule out the development of spontaneous OA, a bilateral arthrotomy without the ACLT was performed as a sham non-OA model.

Injection materials

In this study, we used two HA preparations of different MWs: HA80 (Artz Dispo®, Seikagaku Corp., Tokyo, Japan), with an average MW of 0.8×10^6 Da; and HA600 (Hylan G-F 20, Synvisc®, Genzyme, a Sanofi company, Cambridge, MA), with an average MW of 6×10^6 Da. Both are available for patient use as a medical device loaded with sterile injection material. We used NS (0.9%) as the control.

Treatment regimens

In postoperative week 5, 46 knees from 23 rabbits were injected with NS or HA (HA80 or HA600). NS was injected into the left knees and HA (HA80 or HA600) was injected into the right knees. The sham group (six knees from three rabbits) received bilateral NS injections. The intra-articular injection of 0.3 mL of each material was performed under intravenous anesthesia induced with 0.05 mg/kg phenobarbital sodium using a 26-gauge syringe under sterile conditions. Three weekly injections were administered, and all animals were killed 8 weeks after surgery (1 week after the third injection) with an intravenous lethal dose of phenobarbital sodium. From the OA model, 30 knees were randomly assigned to one of the three treatment groups (10 per group) with NS, HA80, or HA600. From the sham group, five knees were collected randomly from a total of six knees for evaluation.

Macroscopic evaluation

Macroscopic assessment of the knees (five knees per group) was performed in all four compartments of every knee: medial femoral condyle, lateral femoral condyle, medial tibial plateau, and lateral tibial plateau. A solution of India ink diluted with phosphate-buffered saline (1:5) was used to stain the articular cartilage, and the stained specimens were photographed using a high-resolution digital camera. The findings were evaluated according to the following classification: Grade 1 (intact surface), surface was normal in appearance and did not retain India ink; Grade 2 (minimal fibrillation), surface retained India ink as elongated specks or light-gray patches; Grade 3 (overt fibrillation), areas were velvety in appearance and retained India ink as intense black patches; and Grade 4 (erosion), loss of cartilage exposing the underlying bone. Grade 4 was divided further into the following three subgrades: Grade 4a, erosion <2 mm; Grade 4b, erosion ≥ 2 mm and <5 mm; and Grade 4c, erosion ≥ 5 mm[13].

Histopathological evaluation

The distal femur and proximal tibia from each knee (five knees per group) were fixed with 4% phosphate-buffered paraformaldehyde for 24 h, decalcified with 10% ethylenediaminetetraacetic acid (pH 7.4) for 10 days, and then embedded in paraffin. A 5 μm thick sagittal section was cut from the center of each medial femoral condyle. The sections were stained with Safranin-O. The slides were coded before microscopic examination and evaluated by three observers. Degenerative changes to the articular cartilage were assessed quantitatively using the scoring system described by Kikuchi *et al.* (Table I)[14].

Friction test

Fifteen knees from the OA model were used for this test (five per group), and these specimens were used exclusively for this test. The effects of the intra-articular injection of the test substances on joint lubrication were assessed using a pendulum friction tester designed by our laboratory for small samples [Fig. 1]. The knees were resected at the proximal end of the femoral shaft and at the distal end of the tibia and then secured to polyethylene tubes with bone cement. All soft tissues were removed from the joint except the joint capsule and the tendons and ligaments around the knee. The distal end of the tibia of each sample joint was attached to the base plate, and the femoral shaft was attached to the pendulum. The pendulum motion was calculated from two translational displacements by laser displacement sensors (LK-G30, Keyence, Tokyo, Japan), and the angular displacement was calculated by an accelerometer that detected the direction of gravity (100 Hz) (WAA-006, Wireless Technologies, Inc., Tokyo, Japan). The total weight of the pendulum, including its frame, was 40 N in normal sea-level gravity. During the experiments, the joints were kept moist with injections of NS. Based on the linear damping oscillation curve of the pendulum, the frictional coefficient μ was calculated using the following equation: $\mu = L\Delta\theta/4r$, where r is the radius of the rabbit femoral condyle, L is the distance between the pendulum's center of gravity and center of rotation, and $\Delta\theta$ is the average decrease in the amplitude of the pendulum swing per cycle.

Table I
Histopathological scores for the evaluation of cartilage degeneration[13]

Category	+1	+2	+3	+4
Loss of superficial layer	<Slight	Moderate	Focally severe	Extensively severe
Erosion of cartilage	<Detectable	Moderate	Focally severe	Extensively severe
Fibrillation and/or fissures	<Noticeable (<1 very small)	Moderate (1 small)	Marked (2 small or 1 medium)	Extensive (3 small, 2 medium, or 1 large)
Loss of proteoglycans	<Paler stain than control	Moderate loss of Safranin-O stain	Marked loss of Safranin-O stain	Total loss of Safranin-O stain
Disorganization of chondrocytes	Noticeable	Moderate, with some loss of columns	Marked loss of columns	No recognizable organization
Loss of chondrocytes	<Noticeable reduction in cells	Moderate reduction in cells	Marked reduction in cells	Very extensive reduction in cells
Exposure of subchondral bone	<Focal exposure of bone	Moderate exposure of bone	Fairly extensive exposure of bone	Very extensive exposure of bone
Cluster formation	<3–4 small or 1–2 medium	5–6 small, 3–4 medium, or 1–2 large	7 or more medium or 5–6 large	7 or more small, 5–6 medium, or 3–4 large

Dynamic mechanical analysis

After the animals were killed, the joint fluid was aspirated and collected. The samples were centrifuged and stored without preservatives at −80°C. Before testing, the samples were thawed to room temperature. The viscoelastic properties of the joint fluids were measured using a dynamic mechanical analyzer (DMA) (Rheosol-G3000NT, UBM Co. Ltd, Huga, Japan). The rheological properties of the synovial fluids were evaluated at a controlled temperature of 25 ± 1°C. The DMA has two plates, one parallel and one cone-shaped, and the sample was placed between them. The synovial fluid material was subjected to sinusoidal shear forces (strain, stress), and the output signals were recorded. The rheology of the synovial fluid samples was investigated by measuring the steady-state viscosity and small-amplitude oscillatory movements. The small-amplitude oscillatory shear experiments allow the measurement of the unsteady response of a sample and hence the determination of its linear viscoelastic properties[15,16]. The data collected from the DMA are analyzed to yield the shear storage modulus (G'), which gives information about the fluid's elastic character and is related to the energy stored in the fluid during deformation, and the shear loss modulus (G''), which describes the fluid's viscous character and is related to the energy dissipated as heat during flow. The viscoelastic properties of the synovial fluid samples were assessed with a sweeping oscillatory frequency that included the physiological frequencies of knee movement, ranging from 0.01 Hz and 0.1 Hz (slower knee movements, such as occur while at rest and walking, respectively) to 3–4 Hz (more rapid knee movements, such as occur during running).

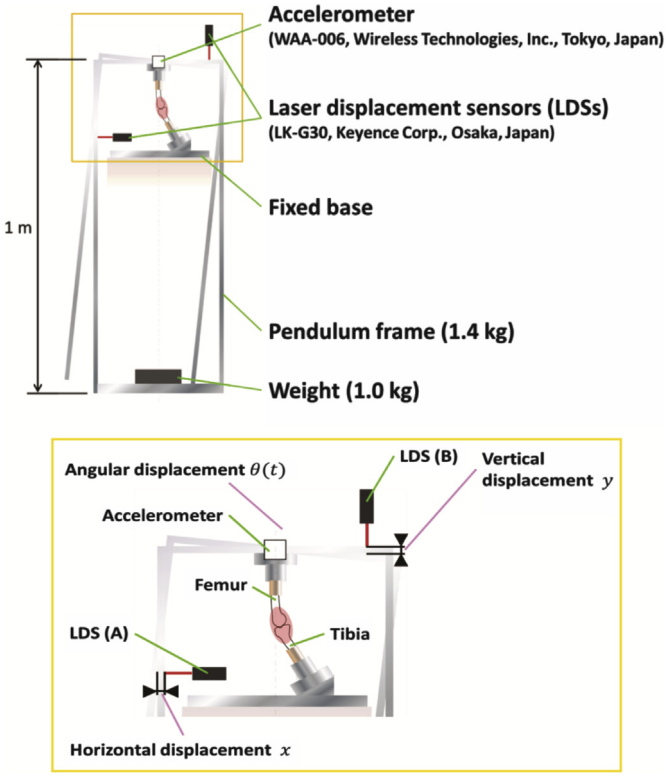

Fig. 1. Illustration of the pendulum friction tester used in this study. The friction coefficient μ was calculated using the equation $\mu = L\Delta\theta/4r$, where r is the radius of the rabbit femoral condyle, L is the distance between the pendulum's center of gravity and center of rotation, and $\Delta\theta$ is the average decrease in the amplitude of the pendulum swing per cycle.

Statistical analysis

The data are presented as mean ± standard deviation (SD). Data were considered independently with the assumption of a Gaussian distribution. The statistical analysis of all data was performed using one-way analysis of variance followed by Tukey's post-hoc test with confidence intervals (CIs) of 95%. *P*-values less than 0.05 were considered significant. All analyses were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Results

Perioperative conditions

All operations were performed smoothly without complications, such as joint sepsis or contractures. When the animals were killed, there was no significant variation in the body weights of the experimental groups, and the complete transection of all anterior cruciate ligaments was confirmed grossly. The joint fluid samples were generally clear or slightly blood tinged, with no gross signs of inflammation or infection; their volumes were in the range of 200–400 μL for samples from HA-injected knees and approximately 100 μL for samples from saline-injected knees.

Gross pathology

Using India ink and a previously described grading system[13], all knees were stained and graded at week 8 after ACLT. All stained knees showed some degree of degenerative changes, ranging from mild to severe, which were, in some cases, accompanied by joint

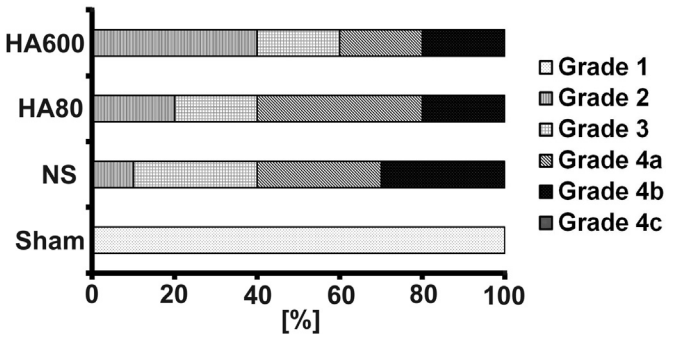

Fig. 2. Macroscopic assessment of the medial femoral condyles from each group 8 weeks after surgery. Cartilage degeneration in the HA600 group was generally less severe than that in either the NS or HA80 group ($n = 5$ per group).

effusion and synovitis. The femoral condyles exhibited more severe changes than the tibial plateaus, which tended to show mild changes. All the samples from the sham group were Grade 1. For the OA model, the NS and HA80 groups showed fairly extensive changes, mainly at the medial femoral condyle, whereas the HA600 group showed milder degeneration. Mild changes (Grade 2: minimal fibrillation) were found in 10% of the NS group, 20% of the HA80 group, and 40% of the HA600 group, whereas severe changes (Grade 4b: erosion $\geq$2 mm and $<$5 mm) were found in 30% of the NS group and 20% of the HA80 and HA600 groups and no samples of Grade 4c were detected [Fig. 2].

Histopathology

Microscopic examination of the articular cartilage showed varying degrees of degenerative change in all the knees from the three groups. The severe changes, such as the loss of the superficial layer, fibrillation/fissures, and the loss of Safranin-O staining, were observed in the NS and HA80 groups [Fig. 3(A) and (B)], whereas samples from the HA600 exhibited less severe changes [Fig. 3(C)]. In the HA80 group, there was a moderate reduction in the severity of cartilage degeneration, whereas in the HA600 group, there was a clear and marked reduction in the severity of the lesions. The loss of Safranin-O staining at the medial femoral condyle was also milder in the HA600 group than in the HA80 group. The overall scores for the HA600 group were significantly lower than those for the NS group ($P = 0.007$). The sham group showed significantly better overall scores compared with the NS ($P < 0.0001$), the HA80 ($P < 0.0001$), and the HA600 groups ($P = 0.0009$). The HA600 group also showed significantly better scores for the loss of the superficial layer and the loss of proteoglycans, whereas only the loss of the superficial layer was significantly better when the HA600 group was compared with the HA80 group ($P = 0.004$). The sham group exhibited better scores for most of the scoring system categories

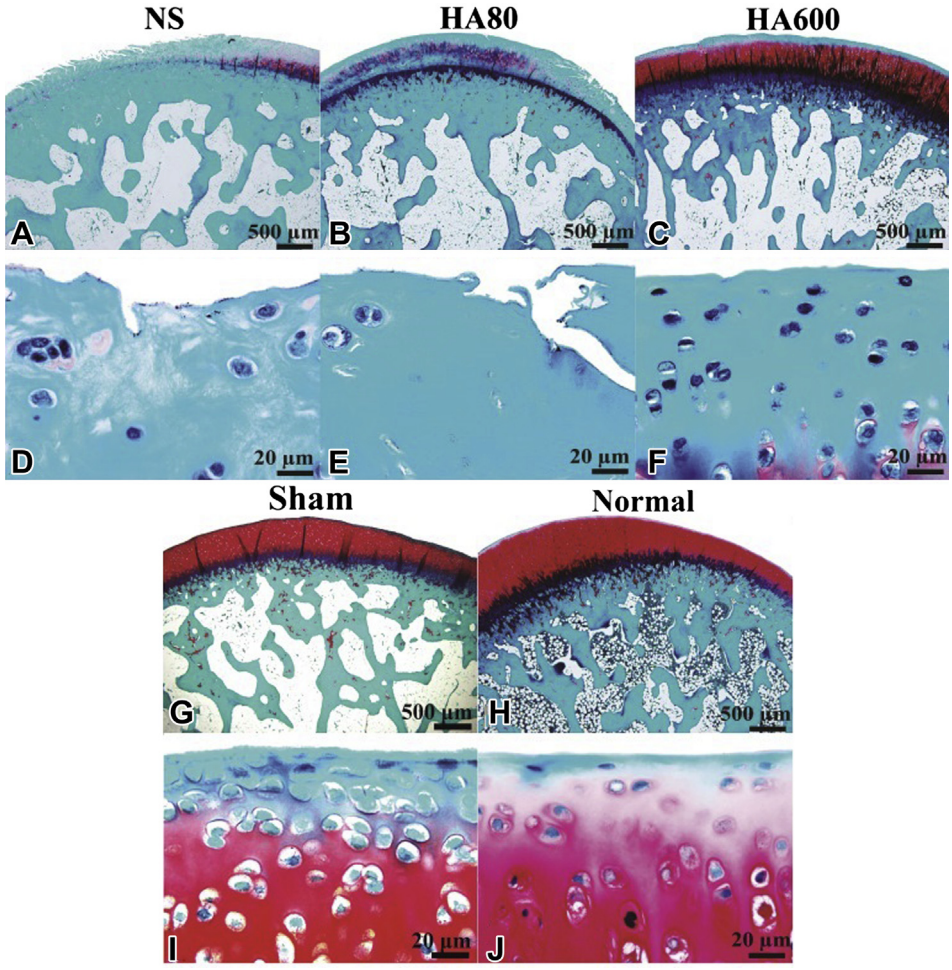

Fig. 3. Histological findings (Safranin-O stain) of the medial femoral condyles from each group 8 weeks after surgery. NS (A, D); HA80 (B, E); HA600 (C, F); sham (G, I); normal knee that had not been operated on (H, J).

Table II
Mean histopathological scores for the medial femoral condyles in each group 8 weeks after anterior cruciate ligament transaction

Category	Sham ($n = 5$)	NS ($n = 5$)	HA80 ($n = 5$)	HA600 ($n = 5$)
Loss of superficial layer	$1.13 \pm 0.18^{*,\S}$	2.53 ± 0.38	2.6 ± 0.43	$1.53 \pm 0.56^{a,b}$
Erosion of cartilage	$1.2 \pm 0.18^{*,\S}$	2.47 ± 0.45	2.4 ± 0.55	1.8 ± 0.61
Fibrillation and/or fissures	$1.07 \pm 0.15^{*,\S}$	2.4 ± 0.72	2.33 ± 0.62	2 ± 0.71
Loss of proteoglycans	$1.2 \pm 0.18^{*,\S,\P}$	2.67 ± 0.33	2.27 ± 0.43	1.93 ± 0.36^{c}
Disorganization of chondrocytes	$1.47 \pm 0.18^{*,\S,\P}$	3.13 ± 0.29	2.23 ± 0.43^{e}	2.47 ± 0.45^{d}
Loss of chondrocytes	$1.07 \pm 0.15^{*,\S}$	2.87 ± 0.61	2.47 ± 0.87	2.07 ± 0.28
Exposure of subchondral bone	$1 \pm 0.0^{\S}$	1.2 ± 0.18	1.8 ± 0.65	1.2 ± 0.45
Cluster formation	$1.2 \pm 0.18^{*,\S,\P}$	2.86 ± 0.38	2.13 ± 0.56	2.33 ± 0.53
Sum	$9.33 \pm 0.24^{\bigcirc,\#}$	20.13 ± 1.28	18.27 ± 2.28	15.33 ± 2.9^{e}

Mean ± SD.
$^{*,\S,\P}P < 0.05$ vs NS, HA80, and HA600, respectively; $^{\bigcirc}P < 0.0001$ vs NS, and HA80; $^{\#}P = 0.0009$ vs HA600; $^{a}P = 0.012$ vs NS; $^{b}P = 0.004$ vs HA80; $^{c}P = 0.018$ vs NS; $^{d}P = 0.04$ vs NS; and $^{e}P = 0.007$ vs NS.

compared with the NS and HA80 groups (Table II). Although the overall scores for the HA600 group tended to be lower than those for the HA80 group, there was no statistically significant difference between the two groups. This was also true when the NS and HA80 groups were compared, and although the HA80 scores tended to be lower than those of the NS group, the differences were not statistically significant. The histological scores (8 = normal to 32 = the severest OA) for the medial femoral condyle are shown in Table II.

Friction test

Knees in the OA model, were tested biomechanically to evaluate the friction coefficients. The friction coefficients were measured using the previously described system while the knees were under 60° flexion. The mean friction coefficient of the HA600 group was significantly lower than that of the NS group ($P = 0.002$), whereas there was no significant difference between the NS and HA80 groups or between the HA80 and HA600 groups, although the values found for the HA600 group tended to be lower than those for the HA80 group [Fig. 4].

Dynamic mechanical analysis

Using the previously described apparatus, the rheological properties of the synovial fluid samples from the three groups (NS, HA80, and HA600) were recorded. Neither the G′ nor the G″ measurements differed significantly between the three groups (Table III).

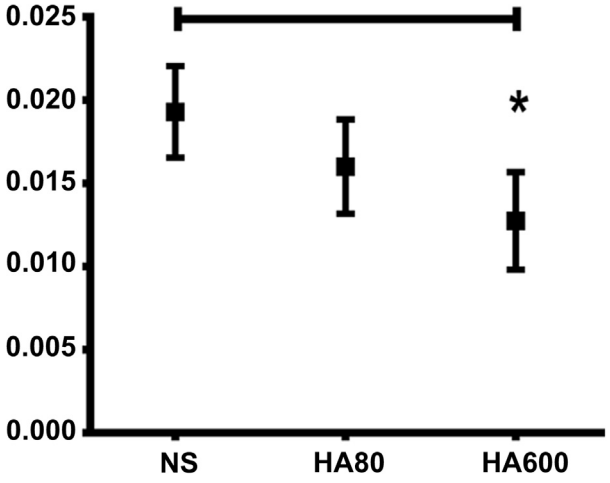

Fig. 4. Mean values for the friction coefficients obtained with the pendulum friction test. Error bars represent the 95% CIs ($n = 5$ per group). $^{*}P = 0.002$ of HA600 vs the NS control.

Discussion

We examined the chondroprotective effects of high-MW CL HA on knee OA induced with ACLT in rabbits. This OA model showed obvious degenerative changes in the articular cartilage 8 weeks after surgery, which resembled those observed in humans. The sham group did not show degenerative changes, thus excluding the possibility of spontaneous OA. Histological analysis showed that the intra-articular injection of HA inhibited OA progression significantly. Within the three treatment groups, less OA progression and better friction coefficients were seen in the HA600 group than in the HA80 and NS groups. These results indicate that intra-articular injections of HA600 have a chondroprotective effect on the articular cartilage.

A number of articles have investigated the effects of different MWs of HA[14,17–20]. Kikuchi *et al.* demonstrated that the injection of low-MW HA (0.8×10^6 Da) did not significantly reduce OA progression compared with NS injection[14]; other reports from Kawano *et al.* and Igarashi *et al.* showed similar results[17,18]. Our histopathological results are consistent with those reports. On the other hand, data from Shimizu *et al.* showed that low-MW HA exhibited a cartilage-protective effect equal to that of high-MW CL HA[20]. In our bilateral ACLT model, OA progression is expected to differ from that in their unilateral model, and the different number of injections, especially for low-MW HA, may affect the outcome. Recent literature reported a new highly CL HA (Variofill®, ADODERM GmbH, Langenfeld, Germany), and the results suggested a better pain relief effect compared with Synvisc®[21,22]. However, we have no data on the analgesic effects of these materials. Future study is needed to investigate the effects of different types of high-MW CL HA.

In the histological analysis, the HA600 group had lower scores for the losses of the superficial layer, proteoglycans, and chondrocytes. Biomechanical testing showed that HA600 yielded significantly lower friction coefficients in the ACLT knees than did saline injections. Therefore, based on the friction test results, we consider high-MW CL HA to act as a chondroprotective factor. A previous article reported that the superficial zone of the articular cartilage does not have special properties that enhance its frictional response[23]. In contrast, several investigators have shown that lubricin (a superficial zone protein, also called proteoglycan 4), which is present both in the superficial layer of the articular cartilage and in the synovial fluid, is very important in joint biomechanics and boundary lubrication[24,25]. Another recent report also showed that supplementation with proteoglycan 4 can restore normal cartilage boundary lubrication to osteoarthritic synovial fluid[26]. Although we did not investigate the lubricin in the cartilage immunohistochemically, our histological findings show clearly the lack of a superficial zone on the surface of the cartilage in all experimental groups, except the normal knee cartilage. The greatest histological difference between the three groups was in the

Table III
Viscoelasticity of normal joint fluid samples from each group compared with the viscoelasticity of the injected HA preparation measured at different frequencies (Hz)

	G′ (Pa) ($n = 5$)				G″ (Pa) ($n = 5$)			
	0.01 Hz	0.1 Hz	1 Hz	4 Hz	0.01 Hz	0.1 Hz	1 Hz	4 Hz
NS	0.05 ± 0.03	0.1 ± 0.07	0.2 ± 0.13	0.26 ± 0.18	0.06 ± 0.04	0.11 ± 0.07	0.15 ± 0.08	0.19 ± 0.08
HA80	0.02 ± 0.02	0.05 ± 0.03	0.14 ± 0.07	0.23 ± 0.15	0.04 ± 0.03	0.1 ± 0.08	0.2 ± 0.17	0.24 ± 0.26
HA600	0.05 ± 0.01	0.1 ± 0.03	0.15 ± 0.06	0.24 ± 0.13	0.07 ± 0.03	0.14 ± 0.06	0.2 ± 0.09	0.25 ± 0.13

Means ± SD ($n = 5$). G′ = shear storage modulus (measuring the fluid's elastic character); G″ = shear loss modulus (measuring the fluid's viscous character); Hz = Hertz; Pa = Pascal.

uppermost layer of the cartilage. In the present study, we defined the uppermost ~100 μm as the superficial layer. We found that the cellular abundance and cellular shape in the uppermost layer in the HA600 group were similar to those in normal unoperated knees, whereas the superficial layer was almost acellular, with no proteoglycans, in the NS and HA80 groups. Seror *et al.* reported that the aggrecan-HA layer is a much better boundary lubricant than HA alone[27], and HA supplementation is also suggested to suppress proteoglycan loss[28]. Of interest, a previous *in vitro* study demonstrated that changes in the MW of HA can affect synovial fluid's cartilage boundary lubricating ability in combination with the physiological levels of proteoglycan 4[29]. A more recent study showed that high-MW CL HA can improve the cartilage integrity and might also stimulate cartilage repair by increasing collagen II and inhibiting interleukin-1β-mediated matrix degradation by reducing matrix metalloproteinases[30]. These data support our histological finding that HA600 preserved the superficial layer and improved the proteoglycan content.

Synovial joint lubrication involves two elements: fluid lubrication and boundary lubrication[31,32]. The viscoelastic behavior of high-MW CL HA may affect the fluid lubrication in the knee joint. The elasticity and viscosity of High-MW CL HA are significantly greater than those of low-MW HA under *in vitro* conditions. However, in our study, measurements of the viscoelasticity of the joint fluid 1 week after the last injection showed no significant differences between the three experimental groups. We consider boundary lubrication to be the main component of the lubrication afforded by our test samples of HA600. The longitudinal rheological evaluation of the joint fluid was limited because the volume of the fluid was small in this rabbit OA model. Changes in the viscoelastic properties of HA and its MW after injection should be clarified in a future study.

This study had several limitations. First, although we are confident that the control injection of NS had little effect on joint lubrication, saline might slightly affect joint lubrication and the rheological characteristics of the synovial fluid. Further, although we repeated the *in vitro* rheological tests on joint fluids at 25°C and 37°C and found no significant differences (data not shown), we conducted no *in vivo* evaluations, which limit the generalizability of our findings. Second, we could not evaluate any changes in the MW of high-MW CL HA *in vivo* because the molecules are very large, making their exact measurement unfeasible. Third, we examined the viscoelasticity measurements at only one time point: 1 week after the third injection. We suspect that measurements should be made at earlier time points to clarify the evolution of differences in viscoelasticity produced by the different injection materials. Finally, the small sample size and the skeletal immaturity of the animal model are also limitation in the current study.

In summary, our findings show that the intra-articular injection of high-MW CL HA is potentially chondroprotective, improving joint lubrication in OA knees induced with ACLT. These attributes should contribute to the retardation of OA progression and consequently modify the pathological processes of OA. This evidence should be useful when selecting HA preparations for intra-articular injection procedures.

Author contributions

All authors contributed to the conception and design of the original study and approved the final submitted manuscript. SE and TF were responsible for the data acquisition and analysis. FS participated in the histological evaluation and data analysis. The article was first drafted by SE, and critically reviewed by TF and NI. ST and MT were responsible for the biomechanical testing. TF takes full responsibility for the integrity of the work.

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Conflict of interest
Teijin Corp., Tokyo, Japan, partially funded this study.

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